1/15/87

JAN 15 1987

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MEMORANDUM

SUBJECT: EPA File Symbol 464-EUP-IT, 6G33306/6H5477. Application for Experimental Use On Aquatic Sites, Petition for Temporary Tolerance in Fish (0.2 ppm) and Request for an Action Level in Potable Water for Garlon 3A (Triclopyr). Accession Nos. 073871, 259680, 073873

Caswell No. 882-I

FROM: William S. Woodrow, Ph.D. WSW 12-16-86
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Robert J. Taylor, PM 25
Fungicide-Herbicide Branch
Registration Division (TS-767C)

THRU: Albin B. Kocialski, Ph.D., Supervisory Pharmacologist
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C) ABK 1/14/87

1/14/87

Petitioner: Dow Chemical Company
Midland, MI 48640

Background:

It would appear that the present request for an aquatic site EUP, a temporary petition for a fish tolerance, and a potable water action level for Garlon 3A (triclophyr) contained in present Dow Chemical correspondence (September 13, 1985) is a [redacted] version of the same request reviewed by Woodrow, May 15, 1986. [redacted]

89844:Woodrow:HED-04:KENCO:12/11/86:2/22/87:bjp:VO

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Recommendations suggested in Woodrow's May 15, 1986 memorandum are reiterated here for clarification:

"Recommendation

1. The Dow Chemical Company request for temporary tolerances of 0.2 ppm for freshwater fish and shellfish, and 0.5 ppm for potable water is toxicologically supported.

   Published tolerances utilized 0.859% of the ADI.

   Total % of the ADI utilized (including 0.2 ppm td. for freshwater fish and shellfish, and 0.5 ppm potable water) = 67.74% of the ADI.

   (See calculations below.)

2. Dow Chemical must submit raw data and results of the current ongoing 2-year rat chronic feeding/oncogenic study for Agency evaluation, prior to consideration of additional permanent tolerances.

3. The EUP label signal word and precautionary statements are satisfactory.

"Calculation of percent ADI utilized:

Fish & shellfish: $0.2 \text{ mg/kg} \times \frac{1.5 \text{ kg}}{\text{day}} \times \frac{1.08}{100} = 0.00324 \text{ mg/day}$

Potable water: $0.5 \text{ mg/kg} \times \frac{1.5 \text{ kg}}{\text{day}} \times \frac{133.33}{100} = 0.99997 \text{ mg/day}$

The Toxicology Branch has no objection to the issuance of this EUP and its attendant request for temporary tolerances. The TMRC would be increased from 0.0129 mg/day (1.5 kg) to 1.0161 mg/day and the percent of the ADI utilized would increase from 0.86% to 67.74%.

There are no regulatory actions pending against this chemical and it is not an RPAR candidate. [redacted]

Present (September 15, 1985) request for EUP, fish tolerance, and potable water action level (464-EUP-IT, 6G3306/6H5477) Garlon 3A

Permanent tolerances for triclopyr and its metabolites are listed in 40 CFR 180.417. Published tolerances include 0.01 ppm in milk, 0.05 ppm in meat, fat, and meat byproducts

(except liver and kidney) of cattle, goats, hogs, sheep, and 0.5 ppm in liver and kidney of cattle, goats, hogs, horses, and sheep (attached).

A temporary tolerance of 0.2 ppm is proposed in freshwater fish and shellfish for triclopyr and its metabolites (triclopyr, 3,5,6-trichloro-2-pyridinyl oxyacetic acid, and its metabolites, 3,5,6-trichloro-2-pyridinol and 2-methoxy-3,5,6-trichloropyridine. This proposed temporary tolerance is based on the following Dow Chemical rationale:

> "In view of the rapid photodecomposition of triclopyr and its metabolites and the fact that this is the maximum possible exposure, fish flesh in unlikely to reach 0.12 ppm in field situations. However, a tolerance level of 0.2 ppm is proposed as a temporary measure."

The temporary tolerance of 0.2 ppm triclopyr in fish is requested for a 2-year EUP for Garlon 3A applied to ponds, lakes, marshes, and reservoirs to control aquatic weeds such as Eurasian Watermilfoil and water hyacinth, and also to control woody plants and broadleaf weeds on stream, canal, and ditch lands.

	Acreage not to exceed	Gallons Garlon 3A	Pounds Active equivalent
EUP 1st year	400	4000	12,000
EUP 2nd year	400	4000	12,000

The Garlon 3A testing will be conducted at not more than 40 acres per year (total not to exceed 400 acres), in the States of Alabama, California, Florida, Georgia, Idaho, New Mexico, Texas, and Washington.

Aquatic weed control with Garlon 3A is to be conducted by Federal, State, or local public agency personnel.

Irrigated Water Treatment

Delay use of treated water for 2 weeks after treatment, or until a Dow-approved assay shows that the water does not contain more than 0.01 ppm triclopyr acid.

Potable Water Treatment

Delay use of treated water for domestic purposes for 2 weeks after treatment, or until a Dow-approved assay shows less than 0.01 ppm.

Fishing

Do not fish treated areas within 24 hours after treatment. Do not trap or dig shellfish within a treated area for 2 weeks after treatment.

Recommendations (Present December 1986 EUP and Temporary Tolerance request)

1. Dow Chemical's request for a temporary tolerance of 0.2 ppm for freshwater fish and shellfish is toxicologically supported.

2. A potable water action level of 0.5 ppm is supported toxicologically, based on the recommendation for a temporary tolerance of 0.5 ppm for potable water in Woodrow's memorandum of May 15, 1986.

3. The following toxicity studies submitted in support of Garlon 3A were reviewed by Dr. Michael Ioannou, of TB

   a. 90-day feeding, rat - using triclopyr (98%).

      Systemic NOEL = 5 mg/kg/day
      Systemic LEL = 20 mg/kg/day
      (Degeneration of proximal tubules in both sexes).
      Doses tested: 0, 5, 20, 50, or 250 mg/kg/day.

      Classification: Supplementary (the test material homogeneity and stability were not reported).

   b. 28-day oral toxicity, rat - using triclopyr (Dowco 233 acid, 99%, and Dowco 233 EGBE, 92.5%).

      Systemic NOEL
      Dowco 233 acid = 100 mg/kg/day
      Dowco 233 EGBE > 300 mg/kg/day
      Dose levels tested: 0, 30, 100, or 300 mg/kg/day
      (both compounds).

Note: NOEL and LEL based upon mortality. No compound or dose related effects noted for other parameters measured.

Classification: Supplementary

c. 21-day dermal toxicity, rats - using Garlon 4 (480 g/L).

NOEL < 5%/kg/day (LDT)
(skin irritation and body weight depression - male rats).
NOEL = 5%/kg/day (LDT)
LEL = 50%/kg/day (MDT)
(Skin irritation and histopathological changes - acanthosis - female rats).
Dose levels tested: 5%, 50%, or 100% at 1 mL/kg/day.

Classification: Core Minimum.

d. 21-day percutaneous absorption, rabbit - using Garlon 4E (480 g/L).

Absorption (intact skin) less than 9% of dose in both sexes - Caused edema, erythema and necrosis - both sexes at both dose levels tested. Dose levels: 125 or 250 mg/kg/day. Repeated treatment with 0.5 and 1.0 mL og 50% aqueous Garlon 4E/kg body wt.

Classification: Supplementary.

e. 21-day percutaneous absorption, rabbit - Garlon 4E (480 g/L).

Absorption through intact skin less than 9% dose, in 24 hours. Caused edema, erythema, necrosis and epithelial hyperplasia. Females only tested at dose level of 500 mg/kg/day. No controls. Repeated treatment with 2.1 mL 50% aqueous Garlon 4E/kg body wt.

Classification: Supplementary.

f. Percutaneous absorption, rabbit - using triclopyr (99% ai).

Absorption through abraded skin less than 1.5% of applied dose in 24 hours. Dose tested: 2 g/kg; only female rabbits tested.

Classification: Supplementary.

g. Percutaneous absorption, rabbit - using 99% ai triclopyr.

Absorption through intact skin less than 1.5% of dose in 24 hours. One dose level tested: 2 g/kg. Only female rabbits tested.

Classification: Supplementary.

h. Mutagenicity - mouse micronucleus test, using 99% ai triclopyr.

Nonmutagenic at dose levels of 28, 90, or 280 mg/kg. Dose levels tested not high enough to indicate cytotoxicity.

Classification: Unacceptable.

i. Metabolism - IV - (urinary excretion), rabbit - using 98% ai triclopyr.

Over 95% of injected INTRAVENOUS dose excreted in urine within 24 hours. Dose level tested: 30 mg/kg. Only two female rabbits used.

Classification: Supplementary.

j. Dietary pharmacokinetics, rat - using 99% ai triclopyr.

Blood concentrations of triclopyr and/or its metabolite pyridinol, increase with increasing dose levels above 50 mg/kg in both sexes. In males there was a disproportionally higher excretion of pyridinol in the urine. Dose levels tested: 0, 5, 20, 50, or 250 mg/kg/day (for 28 days).

Classification: Supplementary.

4. Dow Chemical must submit raw data and results of the current ongoing 2-year rat chronic feeding/oncogenic study for Agency evaluation, prior to consideration of additional permanent tolerances.

5. The 'EUP' label signal word and precautionary statements are satisfactory.

Review of Data:

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Subject: Triclopyr: 13-Week Dietary Toxicity Study in Fischer 344 Rats

Test Material: Triclopyr Technical (GARLON; DOWCO 233), 98% ai (AGR#204229)

Accession No.: 073873

Sponsor: Dow Chemical, U.S.A Midland, Michigan

Testing Facility: Dow Chemical, U.S.A. Midland, Michigan

Study Number: HET K-42085-23

Testing Period: September 1983 to December 1983

Report Submitted to Sponsor: September 1984

Materials and Methods

Fischer 344 male and female rats, 4 weeks old were obtained from Charles River Breeding Laboratory, Kingston, New York and used in this study. Upon arrival, all animals were examined for health status, acclimated to the laboratory conditions for 1 week, weighed, and randomly assigned to 5 different groups, 10 animals/group/sex, of approximately similar mean body weight. Animals were identified with a numbered metal ear tag, placed in individual cages and fed diets intended to provide 0, 5, 20, 50, or 250 mg/kg/day of triclopyr.

The authors reported that earlier tests have shown triclopyr to be stable in the rodent diet for at least 2 weeks and the mixing method (acetone dispersion) produced a homogeneous diet. In the present study, test article homogeneity and stability in the diet were not determined while test article concentrations in the diet were determined three times in males (week 2,8,and 13 on test) and four times in females (week 2, 5, 8 and 13 on test). Dietary concentrations were adjusted weekly for all dose groups based on animal body weight and food consumption (for the first 2 weeks, study adjustments were based on pretest body weights and historical food consumption data).

Clinical Observations for overt signs of toxicity, changes in demeanor, and mortality were carried out routinely (daily). Body weights and food consumption were recorded weekly on all rats.

Clinical chemistry, hematology, and urinalysis parameters were evaluated for each animal. For hematology, blood samples were taken by orbital sinus puncture from each animal after 12 weeks on study and the following parameters were measured:

Packed Cell Volume (PCV)
Hemoglobin (HGB)
Erythrocyte Count (RBC)
Total Leukocyte (WBC)
Differential Leukocyte Count
Mean Cell Volume (MCV)
Mean Corpuscular Hemoglobin (MCH)
Mean Corpuscular Hemoglobin Concentration (MCHC)
Platelet Count (PLAT)

Differential leukocyte counts were performed on the control and top dose group (250 mg/kg/day) only.

For clinical chemistry, blood samples were collected at necropsy from severed cervical blood vessels and the following parameters were evaluated for each animal:

Blood Urea Nitrogen (BUN)
Glutamic Pyruvic Transaminase Activity (SGPT)
Glutamic Oxaloacetic Transaminase Activity (SGOT)
Alkaline Phosphatase Activity (AP)
Glucose (Glu)
Total Protein (TP)
Albumin (Alb)
Globulins (Glob)

Urine was collected after 12 weeks on study and the following urinalysis parameters were measured for each animal:

Bilirubin	pH
Glucose	Protein
Ketones	Urobilinogen
Blood	Specific Gravity

Necropsies were performed on all animals after 13 weeks on study. Prior to scheduled necropsy, animals were fasted overnight, and then anesthetized with methoxyflurane and sacrificed by decapitation. A complete set of tissues shown in table 1 were collected and preserved in neutral, phosphate-buffered 10 percent formalin. Representative sections of tissues were prepared for histological examinations using hematoxylin-eosin stain. All tissues from control and top group (250 mg/kg/day) animals were prepared for histology. Kidney sections from three male rats in each dose group were

also stained with periodic acid-methenamine silver and Masson's trichrome and examined by a veterinary pathologist. The brain, heart, liver, kidneys and testes were weighed and the organ weight to terminal body weight ratios were calculated.

Statistical Evaluation

Body weights, absolute and relative organ weights, clinical chemistry data, appropriate hematology data, and urinary specific gravity were evaluated by Bartlett's test for equality of variances. Based on the outcome of Bartlett's test, exploratory data analysis was performed by a parametric or nonparametric analysis of variance (ANOVA), followed by Dunnett's test or the Wilcoxon Rank-Sum test with a Bonferroni correction for multiple comparisons. Statistical outliers were identified by a sequential test, but routinely excluded only from the food consumption data. Food consumption, white blood cell differential counts, and red blood cell indices are presented with descriptive statistics only (mean and standard deviation).

The nominal alpha levels used and references are as follows:

Bartlett's test (Winer 1971)	$\alpha = 0.01$
Parametric ANOVA (Steel and Torrie 1960)	$\alpha = 0.10$
Nonparametric ANOVA (Hollander 1973)	$\alpha = 0.10$

Dunnett's test (Winer 1971) $\alpha = 0.05$, two-sided
Wilcoxon Rank-Sum test (Hollander 1973) $\alpha = 0.05$, two-sided
Bonferroni correction (Miller 1966)
Outlier test (Grubbs 1969) $\alpha = 0.02$, two-sided

Results

Analysis of the test article concentrations in the diet throughout the study revealed that major deviations from the target concentrations were reported only in three instances as follows:

Dose group	Sex	Week on Test	Percent Change
5 (mg/kg/day)	M	8	-23
	F	5	+18
	F	8	-20

The rest of the values reported for all dose groups in both sexes were between 88 percent and 103 percent of the target concentrations.

Triclopyr stability and homogeneity in the diet were not determined in the present study. However, the authors reported in a separate study (2-week dietary toxicology study in F344 rats, Landry et al.,1983) that triclopyr was shown to be stable in diet up to 2 weeks and also homogeneous in the diet when the acetone dispersion method was used.

The authors reported that there were no overt signs of toxicity in any of the rats that received the test article. Also, no mortality was reported during the study.

Body weight gains in male animals were comparable between controls and animals in the 5 and 50 mg/kg/day dose groups throughout the study. However, body weight gains in the 20 and 250 mg/kg/day dose groups were statistically significantly lower than the controls starting with week 3 on study and lasting until termination. At the end of the study mean body weights of the 20 mg/kg/day group were approximately 7 percent lower than controls while the 250 mg/kg/day group was approximately 13 percent. In female animals there were no statistically significant differences in terminal mean body weights between the treated groups and the controls. The cumulative body weight gain in males was reduced approximately by 8 percent in the 20 mg/kg/day dose group and 17 percent in the 250 mg/kg/day dose group as compared to controls. In females, the cumulative body weight gain was reduced by 7 percent in the 250 mg/kg/day dose group as compared to controls.

Food consumption was consistently lower in male animals of the 250 mg/kg/day dose group as compared to controls. Starting with week 2 on study until termination, food consumption was 9 to 16 percent lower in the 20 mg/kg/day dose group as compared to controls. In females, food consumption throughout the study was similar between control and treated groups.

Several clinical chemistry parameters were found to be statistically significantly different between the treated and control groups in male and female animals. Blood urea nitrogen (BUN) was statistically significantly lower in the 50 and 250 mg/kg/day dose groups of male rats as compared to controls, while in females, BUN was numerically higher in all treated groups as compared to controls (table 2). SGPT was statistically significantly higher in the 250 mg/kg/day dose group males and numerically higher in the females. Total protein was significantly lower in the high-dose group males compared to controls. From the hematology parameters measured, only the platelets concentration in the high-dose group females (250 mg/kg/day) showed a statistically significant decrease compared to controls (table 2).

Urinalysis measurements indicated that the mean urinary specific gravity of male rats of the high-dose groups was statistically significantly lower than in controls. Slight decrease in urinary specific gravity was also reported in female rats of the 50 mg/kg/day dose group. The number of males in the high dose group showing detectable glucose was 5 vs 1 in the control group.

Gross pathology observations did not reveal, according to the authors, any effects of triclopyr in any of the organs/tissues examined.

Histopathological examinations revealed treatment-related changes in the kidneys and liver. In the kidneys of male and female animals, degenerative changes were seen in the descending part of proximal tubules. These changes were present in the 20, 50, and 250 mg/kg/day dose groups (in both sexes) but not in the 5 mg/kg/day dose group. As shown in table 3, the severity of these changes in male animals ranges from very slight to moderate and appears to increase as dose increases. In females, the severity of the degenerative changes ranges from very slight to slight and the most severe changes are seen with the highest dose group (table 3). Additionally, all male animals of the high-dose group (250 mg/kg/day) had renal tubules with decreased amounts of protein absorption droplets. Histopathological changes were seen in the liver of male animals of the high-dose group. These changes consisted mainly of increased eosinophilia (very slight) in the centrilobular hepatocytes. Other changes observed in the liver, kidney, and other tissues were not considered to be treatment related.

Organ weights taken at termination showed in several instances, statistically significant differences between treated and control groups. In males, absolute kidney weight was statistically significantly increased in the 50 mg/kg/day dose group and numerically increased in the 250 mg/kg/day dose group (table 4). Organ weight to body weight ratios (relative organ weight) were also calculated for several tissues. The relative kidney weights in male animals of the 50 and 250 mg/kg/day dose groups were statistically significantly increased compared to the controls. Absolute liver, heart, and testes weights in male animals of the high-dose group were also statistically significantly lower than the controls. Relative brain weights at the 20 and 250 mg/kg/day dose levels were statistically significantly decreased while relative testes weight at 250 mg/kg/day was increased compared to controls. Absolute and relative organ weights in females were found to be comparable between treated and control groups with the exception of the relative kidney weight of the high-dose group which was statistically significantly higher than the corresponding controls (table 4).

TABLE I

TRICLOPYR: 13-WEEK DIETARY TOXICITY STUDY
IN FISCHER 344 RATS

TISSUES TO BE COLLECTED DURING NECROPSY

liver	bone	kidneys
heart	spleen	pancreas
skeletal msucle	salivary gland	brain
pituitary	peripheral nerve	spinal cord
bone marrow	adrenals	stomach
small intestine	cecum	large intestine
mesenteric lymph node	mesenteric tissue	testicles
epididymides	seminal vesicles	coagulating glands
prostate	ovaries	oviducts
uterus	cervix	vagina
urinary bladder	lungs	thymus
mediastinal tissue	mediastinal lymph node	aorta
esophagus	thyroid glands	parathyroid glands
trachea	larynx	skin
mammary gland	eyes	tongue
nasal turbinates	lacrimal glands	Zymbal glands

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Table 2

Effect of Triclopyr on Clinical Chemistry, Hematology and Urinalysis Parameters in F344 Rats

Parameter	Sex	Dose (mg/kg/day) 0	5	20	50	250
Clinical Chemistries						
BUN (mg/dl)	M	17 ± 1	17 ± 2	16 ± 1	15 ± 1*	15 ± 1*
	F	16 ± 1	17 ± 2	17 ± 2	18 ± 2	19 ± 6
SGPT (mu/ml)	M	45 ± 4	41 ± 4	46 ± 6	51 ± 7	60 ± 19*
	F	35 ± 2	35 ± 6	39 ± 7	37 ± 3	45 ± 11
AP (mu/ml)	F	55 ± 8	57 ± 10	53 ± 7	53 ± 7	60 ± 7
TP (g/dl)	M	6.2 ± 0.3	6.2 ± 0.3	6.0 ± 0.2	6.0 ± 0.2	5.8 ± 0.4*
Hematology						
PLAT ($x10^3$/Cumm)	M	781 ± 71	778 ± 78	800 ± 40	836 ± 47	740 ± 77
	F	787 ± 38	803 ± 59	822 ± 47	799 ± 25	689 ± 46*
WBC (10^3x/Cumm)	M	6.5 ± 1.5	6.4 ± 1.0	6.6 ± 0.7	6.7 ± 1.2	6.4 ± 1.2
	F	3.9 ± 0.8	4.6 ± 0.7	4.7 ± 1.1	4.2 ± 0.5	4.0 ± 0.5
Urinalysis						
Specific Gravity	M	1.060 ± 0.006	1.060 ± 0.006	1.060 ± 0.008	1.060 ± 0.008	1.048 ± 0.01
	F	1.046 ± 0.014	1.045 ± 0.013	1.042 ± 0.012	1.038 ± 0.005	1.046 ± 0.00

1/ 10 rats/group
* Statistically significant difference from control mean, $\alpha = 0.05$, two sided.

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Table 3

Histopathological Observations	Sex	Dose (mg/kg/day) 0	5	20	50	250
Kidney						
Degeneration of proximal tubule-descending part, diffuse: - very slight	M	0/10	0/10	2/10	0/10	0/10
- slight	M	0/10	0/10	8/10	2/10	0/10
- moderate	M	0/10	0/10	0/10	8/10	10/10
- very slight	F	0/10	0/10	8/10	10/10	0/10
- slight	F	0/10	0/10	0/10	0/10	10/10
Decreased protein droplets, tubule(s):	M	0/10	0/10	0/10	0/10	10/10
Liver						
Altered tinctorial properties-increased eosinophilia, centrilobular - very slight	M	0/10	0/10	0/10	0/10	10/10

Table 4

Effect of Triclopyr on Absolute Relative Organ Weights in Rats

Dose (mg/kg day)	Sex	Terminal Body Weight, (g)	Organ Absolute(g) and Relative (g/100) Weight									
			Kidney		Liver		Heart		Brain		Testes	
			A[1]	R[2]	A	R	A	R	A	R	A	R
0	M	289±15[3]	2.08±0.12	0.72±0.01	7.70±0.56	2.67±0.09	0.83±0.03	0.29±0.02	1.83±0.04	0.63±0.02	3.03±0.02	1.04±0.03
20	M	270±18*[4]								0.67±0.04*		
50	M	283±18	2.21±0.13*	0.78±0.03*								
250	M	253±12*		0.85±0.03*	6.82±0.29*		0.73±0.05*			0.70±0.03*	2.88±0.13*	1.14±0.06*
0	F	163±9	1.28±0.07	0.78±0.02	4.50±0.38	2.75±0.13	0.55± 0.04	0.34±0.02	1.68±0.04	1.03±0.06		
250	F	157±9		0.86±0.04*								

1/ Absolute organ weight (in grams).
2/ Relative organ weight (grams/100).
3/ N = 10 rats/group (N=9 for testes).
4/ Only values that are statistically significantly different from control values are included.
* Statistically significant difference from control mean, $\alpha = 0.05$.

Discussion

The examination of the analytical results revealed that concentrations of triclopyr in the diet were within acceptable deviation from the target concentrations of 20, 50, and 250 mg/kg/day dose levels. Some variation considered to be outside the acceptable limits was reported for the 5 mg/kg/day dose level for males on week 8 and for females on week 5 and 8 on test. Nevertheless, these variations are not considered to have affected the outcome of this study. Although the stability and homogeneity of triclopyr in the diet has been shown in a separate study, the sponsor should submit that study (Landis et al., 1983, Triclopyr: A 2-week dietary toxicology study in Fischer 344 rats), so that the claim of triclopyr stability and homogeneity can be verified.

No mortality and/or clinical signs of toxicity were reported in rats treated with any of the four dose levels of triclopyr.

Triclopyr administration resulted in reduced body weight gains in all treated groups of male rats compared to controls. The reduction in body weight gain reached statistical significance in the 20 and 250 mg/kg/day dose groups starting approximately the third week on study and continuing to termination. However, the minimal body weight gain reduction reported in the male animals of the 50 mg/kg/day dose group does not allow for a clear dose-response relationship. Examination of the food consumption data indicates that the same dose groups that had reduced body weight gain also had a corresponding reduction in food consumption throughout the study. These results suggest that food efficiency remained unchanged in all groups, and thus does not account for the reduction of body weight gain in the affected groups. Additionally, the lack of any toxicity signs in the treated groups, leads us to believe that the reduced body weight gain in some treated groups is not directly due to triclopyr per se. Possibly the odor of the test article and/or palatability of the diet containing triclopyr might explain these results. This issue is further complicated by the fact that no major adverse effects on body weights were seen in female animals.

Histopathology examinations indicated that triclopyr administration results in the degeneration of the descending proximal tubules of the kidneys in male aand female rats receiving doses of 20, 50, or 250 mg/kg/day of triclopyr (table 3). Kidney weights were significantly increased in male animals of the 50 mg/kg/day dose groups and in male and female animals of the 250 mg/kg/day dose group. Thus, hypertrophy observed in kidneys is greatly supported by the histopathological changes (degenerating tubules) seen in the kidneys of animals

treated with triclopyr at dose levels higher than 5 mg/kg/day. Thus, this reviewer agrees with the sponsor's assessment that effects seen in kidneys (increased weight, degenerative tubules) are directly related to the administration of the test article.

The second histopathological effect observed in the kidneys of male animals treated with 250 mg/kg/day of triclopyr appears to be a secondary effect resulting possibly from altered metabolic changes in these animals due to nutritional changes (less food consumption, lower body weight). Thus, the decreased amounts of protein absorption droplets in the renal tubules could be the result of less formation of α_{2u}-globulins in the liver and thus less amount released into the kidneys. It should be noted here that since this reviewer does not believe that body weight gain reduction is due to a systemic effect of the test article this effect seen in the kidney is not believed to be directly related to test article treatment.

Triclopyr administration also resulted in histopathological changes in the livers of treated animals. Thus, all male animals of the high-dose groups (250 mg/kg/day) had increased eosinophilia of the centrilobular hepatocytes. This histopathological change might have also been the result of altered triclopyr metabolism related to nutrition observed with this treated group. Liver weight for this group (high dose) was statistically significantly lower than controls, apparently related to lower food consumption and lower body weight observed with this group. The altered clinical chemistry values in this group, i.e., lower BUN and total protein and higher SGPT and SGOT might have also resulted from changes in triclopyr biotransformation due to nutritional changes in the male high-dose group.

The slight decrease in mean platelet count in male and female animals of the high-dose groups is not considered biologically significant since there were no indications of morphologic alterations in the hematopoietic system.

Conclusions

The present study has investigated the toxicity of triclopyr in F344 rats when administered in the diet for 13 consecutive weeks at dose levels of 0, 5, 20, 50 or 250 mg/kg/day. Although, body weight gain reduction was seen in male (mostly) and female rats, this effect is not considered to be a systemic effect of triclopyr. The major effect seen in animals exposed to triclopyr was the degeneration of the proximal descending tubules of the kidneys in male and female animals administered either 20, 50, or 250 mg/kg/day of triclopyr. Thus, the NOEL for systemic toxicity is considered to be 5 mg/kg/day while the LEL is 20 mg/kg/day in both sexes.

Classification: Core-Supplementary (Test article homogeneity and stability were not reported)

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87417:Ioannou:C.Disk:KENCO:4/8/86:TAR:MD

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Project: 28-Day Oral Toxicity Study in the Rat with Triclopyr (Study Conducted Under OECD Guidelines)

Test Material: DOWCO 233 Acid (99% ai) and DOWCO 233 EGBE Ester (92.5% ai)

Accession Number: 259680

Sponsor: Dow Chemical, Europe (Rotteram, Netherlands)

Testing Facility: Toxicol Laboratories, Ltd.
Herefordshire, England

Study Number: DNL/1/82

Testing Period: September to October 1982

Report Submitted to Sponsor: February 1983

Materials and Methods:

Male and female COBS rats of the CD strain (purchased from Charles River, UK), weighing 60 to 75 g were used in this study. Upon arrival, animals were examined for their health status, placed in polypropylene cages (five rats of the same sex/cage) and acclimated for 11 days to an air-conditioned room with a temperature of 21 ± 2 °C, relative humidity of 60 ± 10%, and a 12-hour light/dark cycle. Animals were earmarked with a unique number for identification purposes. All animals received rodent diet (SQS No. 1) and water ad libitum throughout this study.

The rats were randomly divided into seven groups, five rats/sex/group and administered orally (by gavage) for 28 consecutive days-0 (group 1), 30 (group 2), 100 (group 3), and 300 (group 4) mg/kg/day of DOWCO 233 acid (99% pure) and 30 (group 5), 100 (group 6), and 300 (group 7) mg/kg/day of DOWCO 233 EGBE ester (92.5% pure). Dosing solutions were prepared daily using 0.25% Gum Tragacanth as the vehicle and administered to the animals within 1 hour of preparation at a volume of 10 mL/kg body weight. Control animals (group 1) received only the vehicle (0.25% Gum Tragacanth).

Animals were observed for signs of toxicity and mortality and the onset, nature, severity, and duration of toxic signs were recorded throughout the study. Bodyweight and food consumption were determined on weekly intervals until termination. Water intake measurements were not carried out.

Clinical chemistry and hematology parameters were evaluated for each animal. Blood samples were taken at termination (after overnight fasting) from the retro-orbital sinus while the animals were under light ether anesthesia. The following parameters were measured:

Hematology

Hematocrit (PCV)
Hemoglobin (Hb)
Erythrocyte Count (RBC)
Mean Cell Volume (MCV)
Mean Cell Hemoglobin Concentration (MCHC)
Total Leucocyte Count (WBC)
Leucocyte Differential Count

Coagulation Tests

Prothrombin Time (PT)

Clinical Chemistry

Blood Urea Nitrogen (BUN)
Glucose (Glu)
Glutamate-Pyruvate Transaminase (SGPT)
Glutamate-Oxaloacetate Transaminase (SGOT)
Total Protein
Sodium (Na)
Potassium (K)
Bilirubin (Bili)
Chloride (Cl)
Calcium (Ca)
Phosphorous (P)
Albumin
Creatinine
γ-Glutamyl Transferase-(γ-GT)

No urinalysis measurements were carried out.

At termination, surviving animals were sacrificed (by unspecified method) and necropsies were performed on all animals. Necropsies were also performed on animals that died on study. Abnormal tissues as well as samples from the following tissues were preserved in 10 percent buffered formalin for subsequent microscopic examinations:

Adrenals	Spleen	Bone Marrow
Heart	Stomach	
Kidney	Testes	
Liver	Thymus	

Thymus and bone marrow smears were taken from females only.

Samples of the tissues listed above were dehydrated, embedded in paraffin wax, sectioned at a 5 micron thickness, and stained with hematoxylin-eosin. Only tissues from male and female animals of group 1 (control), male animals of groups 4 and 7 (high-dose groups), and female animals of groups 3 and 6 (mid-dose

groups) were examined by a pathologist for histopathological lesions. In addition, kidney, liver, and stomach were examined from female animals of groups 4 and 7.

The following organs were weighed and the organ weight to bodyweight ratio was calculated:

Adrenals	Liver
Kidney	Testes

Statistical Analysis:

Wherever possible, data were subjected to analysis of variance followed by comparison of pairs of group means. Statistical significance was calculated from tables.

Results:

The two test compounds used in this study, DOWCO 233 acid and DOWCO 233 EGBE were reported by the sponsor to have purity of 99% and 92.5%, respectively. The sponsor, however, did not specify as to when these compounds were tested for purity and what the impurities were, especially with DOWCO 233 EGBE.

Mortality was reported only with group 4, receiving DOWCO 233 Acid at 300 mg/kg/day (high-dose group). Four out of five females from this group either died (days 11, 13, and 22) or killed in extremis (day 13) and one out of five males was killed in extremis (day 26). The only sign of toxicity observed in surviving treated animals (mainly animals of groups 3, 4, and 7) was salivation. This effect was seen first on day 2 of treatment and persisted throughout the study.

Bodyweight gains were for the most part comparable between treated and control groups. At termination (day 29) the mean bodyweight of the triclopyr-treated groups differed from the control group as follows (reported as percent change from control):

Group	Dose (mg/kg/day)	Males	Females
2	30 (Acid)	-3.3	+3.4
3	100 (Acid)	+7.0	No change
4	300 (Acid)	-7.5	+8.3 1/
5	30 (EGBE)	+2.0	-1.5
6	100 (EGBE)	+5.6	+5.6
7	300 (EGBE)	-7.0	-1.5

1/ Only one animal

Mean food consumption was for the most part comparable between triclopyr-treated and control groups. Higher food

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consumption was reported with group 3 males (10.4%) and group 7 females (10.1%) compared to controls. A somewhat smaller increase in food consumption was seen in group 6 males (5.7%) and in group 4 females (7.9%). Although the food conversion ratio (efficiency index) was variable between the different groups during the last week on study (week 4), the overall food conversion ratio (week 1 through week 4) was comparable between the different groups in both sexes as shown below:

	Total Food Consumption (g/rat)		Food Conversion Ratio	
Group	Male	Female	Male	Female
1	768	556	22.1	13.3
2	752	576	21.1	13.7
3	848	560	22.6	13.4
4	744	600	19.5	15.3
5	780	568	22.4	13.2
6	812	544	22.9	12.0
7	744	612	20.0	11.6

A variety of hematology and clinical chemistry parameters were found to be statistically significantly different between triclopyr treated and control groups for both sexes. As shown in Table 1, however, there is no consistency in these changes and no dose-response relationship was observed with any of the parameters.

From the hematology parameters RBC counts in females of the high-dose group (EGBE, group 7) were statistically significantly decreased compared to controls while the MCV in males and females of the same group were increased. Clinical chemistry parameters that appeared to be affected by triclopyr treatment included BUN in males of the high-dose groups (groups 4 and 7), potassium in males of group 7, SGPT activity in females of group 7, and -GT in males of groups 5, 6, and 7.

Gross pathology results did not reveal major differences between triclopyr-treated and control animals. The most predominant macroscopic lesion reported in most groups was dilated pelvis in the kidneys. As can be seen below, however, this lesion does not appear to be treatment related as no dose dependence was evident:

	Dilated Pelvis	
Group	Male	Female
1	1/5	2/5
2	0/5	4/5
3	2/5	1/5
4	1/5	2/5
5	0/5	2/5
6	1/5	1/5
7	1/5	2/5

TABLE I

Effect of Triclopyr (DOWCO 233 Acid and DOWCO 233 EGBE)
on Hematology and Clinical Chemistry Parameters

		Dose (Mg/kg/day)						
			Acid			EGBE		
		0	30	100	300	30	100	300
Parameter	Sex	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Hematology								
Hemoglobin (g%)	M	14.9±0.8				16.2±0.9*	16.4±1.2*	
	F	15.0±0.9	16.4±0.6*					
MCV (um^3)	M	66 ±1.1		68±0.7*	68±2.2*			69 ± 2.9**
	F	61 ±1.1						64 ± 2.8*
MCHC (g%)	M	35.2±1.7	32.2±0.8*			38.5±1.0*	37.9±3.3*	
~~RCB~~ RBC (10^6/ul)	F	6.5±0.3						5.6 ± 0.3*
Clinical Chemistries								
Bun (mg%)	M	12.1±1.9			14.4±0.7*			14.8±1.7**
	F	15.4±0.9			19.4 1/		17.9±3.1*	
SGPT (U/l)	M	43 ± 7.4	58 ±10.1**	46±9.8	70 ±6.1***	31 ± 5.6*	46 ±12.2	64 ± 7.3***
	F	39 ± 9.4						60 ±10.7***
SGOT (U/l)	M	88 ± 8.1	103±11.0*			71 ±11.6*		
γ-GT (U/l)	M	1.9±1.6				6.2±3.3**	4.4±2.9	5.4±2.4*
Chloride (meg/l)	F	89 ± 3.2					99 ± 7.9*	
Calcium (mg%)	M	9.5±0.1			9.2±0.2*			
Sodium (meg/l)	M	146 ±1.5		143±0.5**				
Potassium (meg/l)	M	4.1±0.1						4.5 ± 0.4**
Total Protein (g%)	F	6.4±0.4		6.0±0.2*				6.0 ± 0.1*

* Significantly different from controls, $p < 0.05$
** Significantly different from controls, $p < 0.01$
*** Significantly different from controls, $p < 0.001$
1/ Only one animal

Histopathological examinations revealed several lesions which occurred randomly in some animals of the control and triclopyr-treated groups. None of these lesions, however, is considered to be of biological significance due to the lack of statistical significance and the absence of a dose-response relationship. Some of these responses (lesions), however, are noted below for the record:

Histopathologigal Lesion	Sex	Group 1	2	3	4	5	6	7
Hydronephrosis (Kidneys)	M	1/5	-	-	1/5	-	-	1/5
	F	2/5	-	0/5	3/5	-	1/5	2/5
Pyelonephritis (Kidneys)	F	0/5	-	1/5	0/5	-	0/5	0/5
Foci of Chronic Inflamatory Cell Infiltration (Liver)	M	0/5	-	-	1/5	-	1/5	0/5

Organ weights were found to be in some cases statistically significantly different between treated and control groups. Table 2 shows that absolute liver weight for female animals was statistically significantly lower than controls in groups 5 and 6 while relative liver weight was lower in groups 2, 3, 5, and 6. In males, lower relative liver weight was observed in group 5 and higher in group 7 compared to controls. Absolute kidney weight in males was statistically significantly higher than controls in group 3 while relative kidney weight was higher in group 4. Statistically significantly higher relative testes weight was observed in group 7 compared to controls.

TABLE 2

Effect of Triclopyr on Absolute and Relative Organ Weight

Dose Group 1		2		3		4		5		6		7	
A	R	A	R	A	R	A	R	A	R	A	R	A	R
	4.9±0.3								4.3±0.3*				5.4±0.5*
.8±1.0	4.8±0.2		4.9±0.2*		4.4±0.2**			8.3±1.1*	4.3±0.3	8.0±1.3*	4.1±0.4		
.6 ± 0.3	0.9±0.1			3.1±0.5*			1.0±0.1*			3.1±1.4*			
	0.9±0.1												1.1±0.1*

A - Absolute
R - Relative

Discussion:

The present study has investigated the toxicity of DOWCO 233 acid and DOWCO 233 EGBE (ester) in rats. Results obtained here indicate that the acid form is relatively more toxic than the ester since the HDT acid of the (300 mg/kg/day) resulted in 80 percent (4/5) deaths of female animals and 20 percent (1/5) deaths of male animals while no mortality was recorded with the ester at the same (300 mg/kg/day) HDT. Thus, based on these findings alone, this reviewer believes that the acid is more toxic than the ester as opposed to the sponsor's conclusions that the toxicity of acid and ester are not different.

Data obtained from bodyweight measurements (mostly terminal body weight) shows that the HDT in males resulted in 7.5 percent and 7 percent lower bodyweights for the acid and ester, respectively, compared to controls. The mid-dose tested (MDT) 100 mg/kg/day for both chemicals resulted in higher body weight (7% and 5.6% in acid and ester, respectively) in male animals. Since the food conversion ratio was approximately the same in all groups, it appears that loss of weight in certain groups was due to lower food intake, possibly due to food palatability.

Data obtained from a variety of other measurements did not reveal any consistent indications as to the effect of triclopyr. Thus, although clinical chemistry, hematology, and organ weight (absolute and relative) measurements were in certain cases statistically significantly different between treated and control groups, no dose-response relationships could be seen with any of the effects. Furthermore, no gross lesions were seen in the treated rats and the histopathological lesions seen in some tissues (liver, kidney, testes) were not statistically significantly different from controls and no correlations could be seen between the lesions and the organ weights or the clinical chemistry and hematology data. The aforementioned observations hold true for both compounds tested, i.e., triclopyr acid and ester.

Conclusions:

The present study has demonstrated that DOWCO 233 acid is more toxic to rats than DOWCO 233 EGBE (ester) based on the observed mortality. Thus, the NOEL for DOWCO 233 acid was considered to be the MDT, i.e., 100 mg/kg/day while the LEL was the HDT, i.e., 300 mg/kg/day (based on mortality incidence). On the other hand, no effects were seen in rats treated with DOWCO 233 EGBE (ester) thus establishing the NOEL and the LEL at higher than 300 mg/kg/day the HDT.

arsification:

The present study is classified as core-supplementary mainly ie to the following deficiencies:

1. The HDT (300 mg/kg/day) for DOWCO 233 ester was not high enough to approximate the MTD.

2. No urinalysis was conducted.

3. No summary tables for histopathological lesions were reported.

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89059:Ioannou:C:DISK:KENCO:6/4/86:6/9/86:DKD:LF

005667

Subject: Subacute (3-week) Dermal Toxicity Study with Garlon 4 in Rats (Note: Study Conducted Under OECD Guidelines)

Test Material: Garlon 4 Herbicide, Formulation Code No. M4021 (Containing Triclopyr, 480 g/L ai)

Accession Number: 259680

Sponsor: Dow Chemical Europe, Horgen, Switzerland

Testing Facility: Netherlands Organization for Applied Scientific Research, TNO.

Project Number: B 84-0436

Testing Period: May 9 to May 30, 1984

Report Submitted To Sponsor: October 1984

Materials and Methods

Male and female SPF rats (Cpb: WU, Wistar random), 8 to 9 weeks old and weighing 200 to 300 g for males or 150 to 200 g for females were obtained from the Central Institute for the Breeding of Laboratory Animals TNO (Zeist, The Netherlands). Upon arrival, the animals were checked for health status and housed in suspended stainless steel cages, five rats/sex/cage. The animals were acclimated for 7 days in a room with a temperature of 23 ± 1 °C, relative humidity of 40 to 70%, a 12-hour light/dark cycle, and 8 to 10 air changes per hour. All animals received a standard diet (name not specified), and water ad libitum.

One day prior to the initiation of the study, the animals (20 males and 20 females) were divided into four groups/sex (based on body weight), and housed individually in stainless steel cages. Cages for each group were colorcoded and each animal was identified by a six-digit number and an earmark. An area of approximately 30 cm^2 in the shoulder region of each animal was shaved using electric clippers. (Application to the shoulder area was made on the premise that this would prevent the rats from ingesting the test compound orally since the treated area remained uncovered and the rats were not restrained.) Shaving was repeated on weekly intervals. The test material, Garlon 4, was used either undiluted (100%) or as 5 or 50% (v/v) dilutions in water. A volume of 1 mL/kg body weight

/day, representing doses of 0.05, 0.5, or 1.0 mL Garlon 4/kg body weight was administered. The vehicle control group was treated with 1 mL water/kg body weight/day. The four test groups used are shown below as follows:

Test Group	No. of rats/group Males	Females
1. Vehicle Control	5	5
2. Garlon 4, 5%	5	5
3. Garlon 4, 50%	5	5
4. Garlon 4, 100%	5	5

All dilutions were prepared fresh daily. The test article was applied to the shaved skin (approximately 10% of the total body surface area) for 3 weeks, 5 days a week. At the end of each daily exposure period (approximately 7 hours/day) the treated skin was rinsed off with water and wiped dry with cellulose tissues (note: the exposed skin was not covered during the exposure period). All animals were inspected daily for general appearance, skin reactions, and signs of toxicity. Any skin effects were recorded for each group on days 1, 2, and 3 and for individual animals at the end of weeks 1, 2, and 3.

Body weight and food consumption measurements were recorded on weekly intervals.

The following hematology parameters were measured by collecting blood samples from the tip of the tail of all rats on day 15 for males and day 16 for females:

- Red blood cells
- Hemoglobin
- Packed cell volume
- Mean corpuscular volume
- Mean corpuscular hemoglobin
- Mean corpuscular hemoglobin concentration
- White blood cells
- Differential white blood cell count
- Thrombocytes

On day 19, after depriving the animals of water (24 hours) and food (16 hours), blood samples were collected from all animals and used in glucose determination. Urine was also collected from all animals during the last 16 hours of fasting and the urine volume and density were determined.

For clinical chemistry measurements, blood samples were obtained from the abdominal aortas of all animals while the animals were under ether anesthetsia, and the following parameters were measured:

- Albumin
- Glutamic-Oxalacetic Transaminase

Alkaline phosphatase	Glutamic-Pyruvic Transaminase
Total bilirubin	Inorganic Phosphate
Calcium	Total Protein
Chloride	Urea
Creatinine	
Sodium	
Potassium	

After collecting the blood samples (day 21) all animals were killed by bleeding (via cannulating the aorta) and subjected to gross pathological examination. The weight of liver, kidneys, adrenals, and gonads from all animals was recorded at sacrifice and the organ weight to body weight ratios (relative organ weights) were calculated. Samples from treated and untreated skin, liver, and kidneys from all animals and adrenals from male animals were preserved in a neutral aqueous phosphate buffered 4% solution of formaldehyde. Histopathological examination was carried out only on tissues dissected from animals (male and female) of the control group and the group treated with 100% Garlon 4. The tissues listed above were embedded in paraffin wax, sectioned at 5/um, stained with hematoxylin-eosin, and examined microscopically.

Statistical Analysis

Data on body weights, red blood cells, thrombocytes, clinical chemistry, and organ weights were evaluated by one-way analysis of (co-) variance followed by Dunnett's multiple comparison test. Food intake and food efficiency figures were evaluated by analysis of variance followed by the L.S.D. test. Total and differential white blood cell counts were analyzed by the Mann-Whitney U-test.

Results

No mortality was reported throughout this study in the control or Garlon 4 treated groups. A variety of dermal effects were observed in most of the animals treated with 50 or 100% Garlon 4 and are presented in table 1. No erythema, edema, scaliness, or incrustation were observed with the control group at the end of weeks 1, 2, or 3 or with the low dose group (5% Garlon 4) at the end of weeks 1 or 2. Slight erythema was observed in all five male rats on the low dose at the end of week 3. All animals of the mid dose group (Garlon 4, 50%) developed slight erythema at all time intervals examined (weeks 1, 2, or 3) with the exception of the male rats that developed well-defined erythema on day 21. All animals of the high dose group (Garlon 4, 100%), with the exception of females at day 7, developed well-defined erythema throughout the study (table 1). The occurrence of edema appeared to be minimal by day 7 in the mid dose group, but by day 14 all male and female animals of this group developed very slight to slight edema which persisted though day 21. With the high dose group slight edema was seen throughout the study (table 1).

Slight to moderate scaliness was observed in all animals of the mid dose group at all time points examined, while moderate to severe scaliness was seen in all animals of the high dose group (table 1). Very slight to slight incrustation was seen in some animals of the mid dose group mostly at day 7 of the study. Moderate to severe incrustation was observed in animals of the high dose group on days 7 and 14 while on day 21 all five male rats and only one female rat had slight incrustation. Other effects reported for the high dose group (only on day 21) included scar tissue (4/5 males and 2/5 females) and decreased hair growth (5/5 males and 3/5 females).

Mean body weights of male rats treated with either 5, 50, or 100% Garlon 4 were statistically significantly lower than the control group at study termination (day 21). For the low and mid dose groups statistical significance was also attained on day 14 while in the high dose group statistical significance was seen on days 7 and 14. In female rats with the exception of the high dose group on day 14, means body weights were comparable between Garlon 4 treated groups and control. Food consumption in male rats was statistically significantly lower than controls in the low and high dose groups on day 7 and all treated and control groups on day 14. No differences in food consumption between Garlon 4-treated and control groups were seen in female rats throughout the study.

The overall Food Conversion Effciency (day 21) was found to be statistically significantly lower than controls in all Garlon 4 treated males and the high dose group females.

TABLE 1

Dermal Effects of Garlon 4 in Male and Female Rats

Dose Level	Sex	Day 7				Day 14				Day 21			
		Erythema	Edema	Scaliness	Incrustation	Erythema	Edema	Scaliness	Incrustation	Erythema	Edema	Scaliness	Incrustation
Control-0%	M	0/5 [1]	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	F	0	0	0	0	0	0	0	0	0	0	0	0
Garlon 4 5%	M	0	0	0	0	0	0	0	0	+ 5 [2]	0	0	0
	F	0	0	0	0	0	0	0	0	0	0	0	0
Garlon 4 50%	M	+ 5	+ 2	++ 5	++ 3	+ 5	+ 5	+ 5	+ 2	++ 5	++ 5	+ 5	+ 2
	F	+ 5	0	++ 5	+ 3	+ 5	++ 5	+ 5	0	+ 5	+ 5	+ 5	0
Garlon 4 100%	M	++ 5	++ 5	+++ 5	+++ 5	+ 5	++ 5	++ 5	++++ 5	++ 5	++ 5	+++ 5	++ 5
	F	++ 5	+ 5	+++ 5	+++ 5	++ 5	++ 5	++ 5	++++ 5	++ 5	++ 5	++ 5	++ 1

1/ Number of animals with specified effect/total number of animals examined.
2/ + denotes severity of specified lesion (see attached legend for full explanation).

1) Degrees of erythema:
- \- no erythema
- \+ very slight erythema
- \+\+ well-defined erythema

2) Degrees of oedema:
- \- no oedema
- \+ very slight oedema
- \+\+ slight oedema
- \+\+\+ moderate oedema

3) Degrees of scaliness:
- \- no scaliness
- \+ slight scaliness
- \+\+ moderate scaliness
- \+\+\+ severe scaliness

4) Degrees of incrustation:
- \- no incrustation
- \+ very slight incrustation
- \+\+ slight incrustation
- \+\+\+ moderate incrustation
- \+\+\+\+ severe incrustation

5) Scar tissue formation:
- \- absent
- \+ slight
- \+\+ distinct

6) Hair growth:
- \- not decreased
- \+ slightly decreased (focal)
- \+\+ slightly decreased (considerable area involved)

Mean relative organ weights in male rats were for the most part similar between Garlon 4-treated groups and control. A statistically significant decrease in relative liver weight was observed with the low dose group (5% Garlon 4) but not with the other treated groups. Thus, no biological significance is attached to this finding. In females, a statistically significant increase in relative kidney weight was reported with the mid and high dose groups with numerical increase seen in the low dose group. These results suggest a dose-response relationship.

From the hematology parameters measured on day 15 in male animals only the white blood cell counts were statistically significantly lower in the low dose group as compared to controls (table 2). In females (parameters measured on day 16), a statistically significant decrease in red blood cell counts, hemoglobin and packed cell volume was observed with the high dose group as compared to controls. Differential white blood cell counts also revealed that the percent lymphocytes in the low dose group was statistically significantly decreased while in the high dose group the percent monocytes were statistically significantly increased when compared to controls. A numerical, but not statistically significant, increase in the percent neutrophils was also observed in females of the low and mid dose groups.

Clinical chemistry measurements, performed on day 21 (day 19 for glucose) revealed that various parameters were statistically significantly different between Garlon 4-treated group and controls as shown in table 2. The statistically significant increase in blood glucose concentration in male rats of the low dose group does not appear to be of any biological significance since such increase was not seen with higher dose levels. Increase in glutamic-oxalacetic transaminase (GOT) activity in males appeared to be dose-related attaining statistical significance in the high dose group. Dose-response relationship was also observed in the increase of glutamic-pyruvic transaminase (GPT) activity in both sexes although the increase did not achieve statistical significance (table 2). Total protein was statistically significantly lower than controls in the high dose group males while albumin was statistically significantly lower in all dose groups. Creatinine was statistically significantly lower in the mid dose group females as compared to controls but not in the low or high dose groups. In males, sodium and calcium levels were statistically significantly different (higher for sodium, lower for calcium) in all Garlon 4-treated groups as compared to controls while chloride was statistically significantly higher in the high dose males.

The two urinalysis parameters measured, i.e., urine volume and density, did not show major differences between the Garlon 4-treated and control groups in either sex.

TABLE 2

Effect of Garlon 4 on Hematology and Clinical Chemistry Parameters

rameter	Sex	Dose (% Garlon 4) 0	5	50	100
natology					
ite blood cells (10E9/L)	M	16.8±0.7	13.0*±0.8 1/	14.7±1.1	16.6±0.7
l blood cells (10E12/L)	F	7.0±0.2	7.0±0.2	6.8±0.1	6.0**±0.1
noglobin (mmol/L)	F	8.9±0.2	8.8±0.2	8.8±0.1	8.0**±0.1
cked cell volume (L/L)	F	0.458±0.007	0.450±0.007	0.445±0.005	0.420***±0.005
inical Chemistry					
ucose (mmol/L)	M	3.2±0.1	3.7**±0.0	3.3±0.1	3.1±0.1
T (U/L)	M	57.8±2.7	60.9±1.8	67.0±2.6	70.9*±5.0
T (U/L)	M	43.3±3.4	45.6±1.9	47.1±2.6	49.4±4.6
	F	43.4±2.4	47.2±4.0	49.7±6.2	51.4±6.1
tal protein (G/L)	M	60.2±0.3	59.7±0.7	58.6±0.5	57.2**±0.6
bumin (G/L)	M	33.7±0.6	30.3**±0.6	28.7**±0.7	30.0**±0.4
eatinine (umol/L)	F	61.8±1.3	60.8±1.2	56.8*±1.2	60.2±0.9
dium (mmol/L)	M	145.7±0.4	148.0**±0.4	148.4**±0.5	147.9**±0.4
lcium (mmol/L)	M	2.39±0.02	2.29*±0.03	2.25**±0.02	2.26**±0.02
loride (mmol/L)	M	97.1±0.4	97.2±1.3	97.4±0.1	101.5**±0.6

1/ Statistically significantly different from control
*P < 0.05
**P < 0.01

Gross pathology performed on all animals at necropsy did not reveal any statistically significant differences in macroscopic lesions between Garlon 4-treated and control groups, other than the skin changes discussed earlier in this section (see table 1).

Histopathological examination revealed a variety of lesions in the skin of treated animals but not in any of the other organs examined. Table 3 (abstracted from the original report) shows the incidence of different skin lesions in treated and untreated groups of both sexes. The incidence of acanthosis in both sexes was statistically significantly higher in the mid (50% Garlon 4) and high (100% Garlon 4) dose groups as compared to the corresponding controls (table 3). The total incidence of subepithelial mixed inflammatory-cell infiltration was also statistically significantly higher in the mid (5/5) and high (5/5) dose group males and in the mid (4/5) and high (5/5) dose group females compared to controls (0/5). Other skin lesions observed in treated animals that were numercially but not statistically significantly higher than controls included: Focal follicular mixed inflammatory-cell infiltration (slight) (0/5, 2/5, and 1/5 in male and female controls, high dose males and high dose females, respectively); epidermal necrosis (0/5 and 1/5 in control and high dose male group, respectively); crusting (0/5 and 2/5 in control and high dose male group, respectively).

Discussion

The present study has investigated the dermal toxicity of Garlon 4 in male and female rats. Several compound-related effects were observed mostly in animals treated either with 50 or 100% Garlon 4. Garlon 4 causes slight skin irritation to male rats; at the 5% dose level, slight to moderate skin irritation to both sexes of rats at the 50% dose level, and severe skin irritation in both sexes at the 100% dose level. It appears that skin irritation increases with dose and number of applications and the male rats appear to be slightly more susceptible than female rats.

Body weight gains on day 21 were statistically significantly lower in male rats in all Garlon 4-treated groups as compared to controls. At the same time food consumption by the same Garlon 4-treated males as statistically significantly lower than controls. The overall food conversion efficiency of these animals was also statistically significantly lower than controls. These results suggest that for male rats, Garlon 4, probably because of its irritant effect on the skin, decreases the animal's food intake. The lower food conversion efficiency reported for these animals cannot be explained with the available data (i.e., none of the clinical signs reported can account for this finding).

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TABLE 3

3-WEEK DERMAL TOXICITY STUDY WITH CARLON 4 IN RATS
SUMMARY OF HISTOPATHOLOGICAL OBSERVATIONS

LESIONS	TREATMENT	INCIDENCE OF LESIONS (NUMERIC) MALES CONTR.	5%G4	50%G4	100%G4	FEMALES CONTR.	5%G4	50%G4	100%G4
SKIN/SUBCUTIS:		(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
TREATED SKIN:									
no abnormality detected		5	5			5	4		
acanthosis									
	total incidence			5**	5**		1	5**	5**
	very slight			4*			1	2	
	slight			1	2			3	2
	moderate				3				3
subepithelial mixed inflammatory-cell infiltration									
	total incidence			5**	5**			4*	5**
	very slight			5**	3			4*	3
	slight				2				1
	moderate								1
focal follicular mixed inflammatory-cell infiltration									
	slight				2				1
epidermal necrosis					1				
crusting					2				
UNTREATED SKIN:									
no abnormalities detected		5	5	5	5	5	5	5	5

Figures in brackets represent the number of animals from which this tissue was examined microscopically
The absence of a numeral indicates that the lesion specified was not identified
Significance of differences in a pairwise (Fisher's) test between each treatment and control incidence: * P<0.05, ** P<0.01

Hematological changes that could be attributed to Garlon 4 application were observed only in the high dose group females as a statistically significant decrease in red blood cell counts, hemoglobin, and packed cell volume. These results might suggest some interference of Garlon 4 with the hemopoietic system.

Regarding clinical chemistry parameters it appears that in male rats a weak dose-response relationship was seen in the increase of GOT and GPT activities in plasma. However, the biological significance of this trend cannot be established since no histopathological changes (lesions) were seen in the livers of the treated animals. It is possible that these changes (increased GOT and GPT activities) may be associated with the nutritional status of the animals (depressed food intake, low conversion efficiency). The decrease in albumin (in male rats) and hence in total protein (weak dose-response) may also be associated with the nutritional status of the animals since no histopathological changes were seen in the liver or kidneys which could explain this decrease. Statistically significant increase in sodium and decrease in calcium in all male treated groups were attributed by the authors to the low food intake and low food efficiency by these animals rather than any direct effect of Garlon 4 on these parameters. The statistically significant increase in chloride in male animals of the high dose group cannot be explained by the nutritional status of the animals and its biological significance is unknown.

A dose-response relationship was observed in the increase of relative kidney weight in female animals. However, the toxicological significance of this finding cannot be established since this change was not associated with any treatment-related histopathological lesions. Furthermore, the two urine parameters measured (volume and density) were not different between the Garlon 4-treated group and controls.

The histopathological lesions (acanthosis and subepithelial mixed inflammatory-cell infiltration) seen in the skin of male and female animals of the mid and high dose groups are considered to be the direct effect of Garlon 4 treatment. The high incidence of acanthosis (100% or male and female animals), which is the result of proliferative changes of the treated skin, suggests that Garlon 4 is a strong topical skin irritant.

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Conclusions

The results of the present study indicate that Garlon 4, when applied on the skin for 21 consecutive days, results in a variety of effects in male and female rats. In male rats, the NOEL is considered to be lower than 5% the lowest dose tested based on its adverse effect on body weight and skin irritation. In female rats the NOEL is considered to be the 5% dose level and the LEL the 50% dose level based on skin irritation and histopathological changes (acanthosis).

Classification

The present study is classified as Core-Minimum (inerts used in the formulation and/or impurities were not supplied to the Agency).

005667

09061:Ioannou:HED-24:KENCO:6/13/86:6/17/86:sj:LF

Project: Subacute (3-Week) Percutaneous Absorption Study with Garlon 4E in Albino Rabbits. Part II: Repeated Treatment with 2.1 mL 50 percent Aqueous Garlon 4E/kg Body Weight. (Note: Study probably conducted under OECD Guidelines.)

Test Material: Garlon 4E (4021) Herbicide Containing 480 g Triclopyr/L; Lot No. MMO 414021.

Accession No: 259680

Sponsor: Dow Chemical Company Ltd., Norfolk, U.K.

Testing Facility: Netherlands Organization for Applied Scientific Research, TNO.

Report Number: V81.511/212063 Project No. 981/2063

Testing Period: September 1981

Report Submitted to Sponsor: December 1981

Materials and Methods:

Two young adult female New Zealand White albino rabbits weighing 2.91 and 2.95 kg, respectively, were used for the study (age and source of animals was not reported). The animals were kept in individual cages in a room with a temperature of 18±2 °C and had free access to standard laboratory rabbit diet (name of diet not specified) and tap water. The day prior to the initiation of treatment approximately 10 percent of the body surface of each animal (back and flanks) was shaved using electric clippers. On days 1, 7, 14, and 21 the animals were weighed, put in restraining boxes and their bladders emptied using a catheter. Garlon 4E (a clear brown liquid) was diluted (1:1) with water and applied to the uncovered shaved skin at a volume of 2.1 mL/kg body weight. The animals were kept in restraining boxes for 24 hours without food or water and at various intervals their bladders were emptied using a catheter. The urine samples were stored in a freezer until analyzed for triclopyr content using the EC-GLC procedure. The recovery of added triclopyr was determined to be greater than 93 percent.

On days 2 to 4, 8 to 11, and 15 to 18 the animals were treated with diluted Garlon 4E in their individual cages and had free access to food and water. To prevent animals from licking the chemical from the treated skin all animals wore neck collars during exposure. Garlon 4E was applied to the skin on a daily basis, 5 days a week for 3 weeks at a dose equivalent to 500 mg triclopyr/kg body weight.

During the study the animals were observed for clinical signs of toxicity and mortality. At the end of the study the rabbits were killed under Euthesate anesthesia by exsanguination. Samples of the treated skin were collected and fixed in a 4 percent neutral phosphate-buffered formaldehyde solution for histological examination.

Results:

The present study has investigated the dermal absorption of Garlon 4E in rabbits after repeated exposure for 21 days at a dose equivalent to 500 mg triclopyr/kg body weight/day. According to the authors, at the early stages of exposure the animals developed moderate erythema and slight edema of the treated skin. After 1 week of treatment and throughout the rest of the study both animals showed stiff and necrotic skin. Body weight remained unchanged in the course of the study (Table 1). Gross pathological examination of the treated skin revealed scaliness and necrosis (Table 2). Microscopic examination of the skin revealed several treatment-related changes in both animals consisting mainly of slight focal or diffuse acanthosis and moderate focal or diffuse hyperkeratosis (Table 2). These changes were characterized by epithelial hyperplasia and inflammatory response.

The amount of Garlon 4E absorbed from the skin (as determined by the amount of triclopyr excreted in the urine) was estimated at four time points (day 1, 7, 14, and 21) during this study. As shown in Table 1, the excretion of triclopyr into the urine, as percent of the total dose applied varies with the animal and the number of exposures. On day 1, 6.7 percent of the dose is excreted is the urine and on day 7, 13.9 percent.

The higher excretion after repeated exposure (day 7) might be due to increased absorption resulting from skin injury. On day 14, 9.9 percent of the dose was excreted in the urine while on day 21, only 3.7 percent was excreted. The low excretion reported for day 21 might be the result of decreased absorption due to extensive necrosis of the treated skin (thus forming a barrier for Garlon 4E absorption).

The average excretion of triclopyr in the urine as percent of the total dose applied to the skin was 8.5 percent. This figure is comparable to the figure (8.9%) obtained from a similar study (Report No. V81.510) where Garlon 4E (50% dilution) was applied to the skin of female rabbits for 3 weeks at volumes of 0.5 to 1.0 mL/kg/day (equivalent to a dose of 125 or 250 mg triclopyr/kg body weight/day, respectively.

4:

Conclusions:

Under the conditions of the present study, dermal application of 50 percent aqueous dilution of Garlon 4E at 2.1 mL/kg/day (equivalent to 500 mg/kg/day) to female albino rabbits, results in approximately 8.5 percent excretion of triclopyr in the urine within 24 hours. Repeated dermal exposure appears to have an effect on triclopyr excretion in the urine (and hence absorption from the skin) depending possibly on the state of the exposed skin. Exposure to Garlon 4E also results in various skin effects including erythema, edema, scaliness, necrosis, and epithelial hyperplasia.

Classification:

The present study is classified as Core-Supplementary because of the following defficiencies:

1. Only two animals were used.
2. The authors did not estimate the total recovery of Garlon 4E by taking into account quantities of triclopyr retained in the tissues, excreted in feces, or remaining on the application site.
3. The stability of Garlon 4E in water at room temperature for up to 24 hours was not reported.
4. No control animals were used.
5. No information was reported on possible triclopyr metabolites present in urine.

The above deficiencies were also seen in a similar study (CIVO Report V81.510) where female rabbits were exposed (for 3 weeks) to 50 percent Garlon 4E at dose levels of 125 or 250 mg/kg/day (or a volume of 0.5 or 1.0 mL/kg/day).

TABLE 1. URINARY EXCRETION OF TRICLOPYR BY TWO FEMALE RABBITS AFTER REPEATED DERMAL APPLICATION OF GARLON 4E, 1:1 DILUTED WITH WATER

rabbit no.	body weight kg	dosage ml/kg body wt.	triclopyr equivalents applied mg	amount of triclopyr excreted mg	amount in per cents of dose applied %
day 1					
302	2.91	2.1	1464	71.2	4.9
303	2.95	2.1	1489	124.6	8.4
day 7					
302	2.80	2.1	1416	136.7	9.7
303	3.01	2.1	1512	272.2	18.0
day 14					
302	2.84	2.1	1440	140.7	9.8
303	3.05	2.1	1536	154.0	10.0
day 21					
302	2.83	2.1	1416	49.0	3.5
303	2.85	2.1	1440	55.5	3.9

TABLE 2. TREATMENT-RELATED PATHOLOGY; INDIVIDUAL DATA

Dose (ml/kg)	2.1	
Animal number	20K	10K
Macroscopic examination		
Skin: NECROSIS	X	X
SCALY	X	
Microscopic examination		
Skin		
1) SLIGHT FOCAL OR DIFFUSE ACANTHOSIS	X	X
2) MODERATE FOCAL OR DIFFUSE HYPERKERATOSIS	X	X
3) INCREASED NUMBER OF POLYMORPH-NUCLEAR INFLAMMATORY CELLS IN THE UPPER PART OF THE DERMIS	X	

Subject: Subacute (3-Week) Percutaneous Absorption Study with Garlon 4E in Albino Rabbits. Part I: Repeated Treatment with 0.5 and 1.0 mL of 50 Percent Aqueous Garlon 4E/kg Body Weight. (Note: Study probably conducted under OECD Guidelines.)

Test Material: Garlon 4E (480 g triclopyr/L).

Accession Number: 259680

Sponsor: Dow Chemical Company Ltd., Norfolk, U.K.

Testing Facility: Netherlands Organization for Applied Scientific Research, TNO.

Report Number: Vbl.510/2.2063 Project No. 891/2063

Testing Period: September 1981

Report Submitted to Sponsor: November '981

Materials and Methods:

Four female New Zealand White albino rabbits weighing 2.7, 2.9, 3.2, and 2.9, respectively, were used for this study (age and source of animals were not reported). The animals were kept in individual cages in a room with a temperature of 18±2 °C and offered standard laboratory diet (name of diet not specified) and water ad libitum. The day before the initiation of treatment approximately 10 percent of the body surface of each animal (back) was shaved using electric clippers. On days 1, 7, 14, and 21 all animals were weighed, placed in restraining boxes, and their bladders emptied using a catheter. The animals were then divided into two groups, two animals/group and their uncovered shaved skin was exposed to either 0.5 mL of 50 percent solution of Garlon 4E/kg body weight (group I) or 1 mL of 50 percent solution of Garlon 4E/kg body weight (group II). The animals were kept in restraining boxes for 24 hours without food or water and at various intervals during this period their bladders were emptied using a catheter. The urine samples collected from each animal were analyzed later for triclopyr content using the EC-GLC procedure. Triclopyr recovery (spiked samples) was determined to be greater than 93 percent.

All animals were returned to their individual cages where they were treated with the corresponding volume of 50 percent Garlon 4E on days 2 to 4, 8 to 11, and 15 to 18. During these days all animals wore a neck collar to prevent

them from licking the test material from the treated skin. The animals were treated daily, 5 days a week for 3 weeks with Garlon 4E at doses corresponding to 125 (group I) or 250 mg (group II) triclopyr/kg body weight.

Throughout the study, all animals were observed for mortality, toxicity, and local skin reactions. At termination of the study the animals were killed under Euthesate anesthesia by exsanguination. Samples of the treated skin were collected and fixed in a 4 percent neutral phosphate-buffered formaldehyde solution for histological examination.

Results:

The present study has investigated the dermal absorption of Garlon 4E in rabbits after repeated applications for 21 days at two dose levels. The authors reported that a variety of clinical signs of Garlon 4E toxicity were seen in all treated animals. After the initial exposure period, animals of both groups showed slight to moderate erythema and slight edema. At later exposure periods and by day 21, surviving rabbits showed the following dermal effects:

> Group I (0.5 mL of 50% Garlon 4E/kg/day) - Scaliness and slight necrosis.
>
> Group II (1.0 mL of 50% Garlon 4E/kg/day) - Scaliness, slight to distinct necrosis and some decreased hair growth.

Note: One rabbit from group I was killed on day 4 of the study after exhibiting declining health, probably due to pneumonia, according to the authors.

The amount of Garlon 4E absorbed from the skin as determined by the amount of triclopyr excreted in urine was estimated at four time points (day 1, 7, 14, and 21) during the study for both dose groups. Data presented in Table 1 (abstracted from the original report) show that within 24 hours after exposure (day 1) 10 percent of the total dose applied is excreted in the urine of group I rabbits and 7.9 percent excreted in the urine of group II rabbits. At later time points (day 7, 14, and 21) the percent triclopyr excreted in the urine was consistently lower in group I (only 1 animal) and consistently slightly higher in group II rabbits. The overall (average of the 4 time points) triclopyr excretion in urine was approximately 8 percent and 9 percent of the total dose for group I and group II animals, respectively. Those results suggest that for group II animals, repeated dermal exposure does not have a significant effect on the amount of

triclopyr absorbed and excreted in the urine. For group I animals, although repeated exposure appears to lower significantly the amount excreted in the urine (day 7 and 14), these results are based on data derived from a single animal and thus their significance is not known.

As with earlier dermal absorption studies with triclopyr (Report No. V81.322; Report No. V81.422) the authors equate the percent triclopyr excreted in the urine to the percent of the total dose absorbed through the skin. This reviewer does not agree with this assumption since the authors have not proven this equality (also see comments in aforementioned earlier dermal absorption studies).

Based on data presented here, the authors conclude that dermal absorption of Garlon 4E (2-butoxyethyl ester of triclopyr) is approximately sevenfold higher than that of an aqueous suspension of triclopyr. Although there might be some validity in this conclusion (when based strictly on the percent total dose excreted in urine) the authors have yet to explain how, in the earlier studies (Report No. V81.322 and V81.422), they were able to apply over 30 mL of triclopyr suspension on a small area of the skin without major losses (runoff). It is the opinion of this reviewer that most of the 2000 mg triclopyr applied to the skin (as 30 mL suspension) never came in full contact with the skin and thus had no chance to be absorbed. Thus, the problem of applying significantly different volumes to the skin between this and the previous studies (different protocols) and the failure of the authors to account for the total recovery of the applied dose (in all studies) make their conclusions questionable.

Gross pathology examination (Table 2) did not reveal any treatment-related changes other than skin irritation described above. Histopathological examination of the treated skin revealed a variety of treatment-related changes in both dose groups as seen in Table 3. These changes included: acanthosis (slight in low dose, moderate in high dose group); hyperkeratosis (slight in low dose, moderate in high dose group); parakeratosis (slight in high dose group); edema in the dermis (slight in both dose groups); and crusts (in high dose group). Note: These changes seen on the treated skin (macroscopic and microscopic) cannot be evaluated fully since no vehicle control values have been supplied by the authors.

Conclusions:

Data presented in this study indicate that:

1. The percent of the total dose absorbed from the skin and excreted in the urine as triclopyr was approximately the same between the two dose groups and accounted for 8 to 9 percent of the applied dose.

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2. Repeated dermal exposure to Garlon 4E (either low or high dose level) did not change the dermal absorption.

3. Repeated dermal exposure to Garlon 4E results in slight to moderate erythema and slight edema, as well as epidermal degeneration and epithelial hyperplasia.

Classification:

The present study is classified as Core-Supplementary (for deficiencies discussed above).

TABLE 1. URINARY EXCRETION OF TRICLOPYR BY RABBITS AFTER REPEATED DERMAL APPLICATION OF A 50 % AQUEOUS DILUTION OF GARLON 4E

rabbit no.	body weight kg	dosage ml/kg body wt.	triclopyr equivalents applied mg	amount of triclopyr excreted mg	amount in per cents of dose applied %
	day 1				
379	2.66	0.5	312	35.6	11.4
380	2.94	0.5	360	31.0	8.6
382	3.18	1.0	768	50.6	6.6
384	2.94	1.0	696	57.8	8.3
	day 7				
379	2.47	0.5	288	18.3	6.4
380	-[1]	-	-	-	-
382	2.92	1.0	696	66.6	9.6
384	2.70	1.0	648	75.5	11.7
	day 14				
379	2.87	0.5	336	17.5	5.2
380	-	-	-	-	-
382	3.07	1.0	744	74.5	10.0
384	2.92	1.0	696	54.7	7.9
	day 21				
379	3.05	0.5	360	29.0	8.1
380	-	-	-	-	-
382	3.10	1.0	744	93.4	12.6
384	2.90	1.0	696	48.8	7.0

[1] - = killed on day 4

TABLE 2. HISTOPATHOLOGY: INDIVIDUAL DATA[1)]

Dose (ml/kg)	0.5		1.0	
Animal number and sex	1♀	2♀	3♀	4♀
Killed during the experiment on day:		4		
Macroscopic examination				
Skin: SCALY	X		X	X
RED DISCOLOURED		X		
Abdominal cavity: ascites		X		
Spleen: pale		X		
Kidneys: pale		X		
Liver: pale		X		
Lungs: evidence of inflammation		X		
Microscopic examination				
Skin				
1) ACANTHOSIS a) slight	X			
b) moderate			X	X
2) HYPERKERATOSIS a) slight	X			
b) moderate			X	X
3) SLIGHT PARAKERATOSIS			X	
4) SLIGHT EDEMA IN THE DERMIS	X		X	X
5) CRUSTS; consisting of cellular debris			X	

1) Treatment-related changes are written in capitals

Subject: Study on the Percutaneous Absorption of Triclopyr by the Rabbit. Part I: Single Treatment of Abraded Skin. (Note: Study probably conducted under OECD Guidelines.)

Test Material: Triclopyr, 99% ai (3,5,6-trichloro-2-pyridyloxyacetic acid).

Accession Number: 259680

Sponsor: Dow Chemical Company Ltd., Norfolk, U.K.

Testing Facility: Netherlands Organization for Applied Scientific Research, TNO.

Report Number: V81.322 Project No. B81/1733

Testing Period: Not specified (possibly April 1981)

Report Submitted to Sponsor: August 1981

Materials and Methods:

Two female New Zealand White albino rabbits, weighing 2.8 and 2.9 kg were used in this study (the age and source of animals were not specified). Prior to the initiation of the study the animals were offered standard laboratory diet (name not specified) and water ad libitum. The day before the start of the experiment, approximately 20 percent of the body surface was shaved with electric clippers (trunk area) and just prior to the application of the chemical the shaved skin was abraded using a sterile injection needle and then wrapped with polyethylene foil. The animals were then placed in restraining boxes and their bladders emptied using a catheter. Triclopyr, a grey-white crystalline powder, 99% pure (AGR 134812) was dissolved in demineralized water to give a 20 percent (w/v) suspension.

The suspension was then injected through the polyethylene foil onto the shaved abraded skin. A total of 28 or 29 mL of the 20 percent suspension was used for each animal corresponding to a triclopyr dose of 3 g/kg body weight (and a volume of 10 mL/kg body weight). After application, the animals were kept in the restraining boxes for 24 hours without any food or water. At various intervals, urine was removed from the bladders using a catheter and analyzed for triclopyr content using EC-GLC technique. Triclopyr recovery (from spiked samples) was reported to be greater than 93 percent.

Results:

The present study has investigated the dermal absorption of triclopyr in rabbits when applied to the abraded skin at a level of 2 g/kg body weight. The data presented here (Table 1) indicate that, based on the amount of triclopyr excreted in the urine, the rate of dermal absorption is very slow throughout the 24-hour period resulting in a total excretion in the urine of approximately 1.1 percent of the applied dose. The authors are going on the assumption that over 90 percent of the amount absorbed is excreted in the urine within 24 hours. This is based on an earlier study (Report Vol.3I2) where over 90 percent of triclopyr injected intravenously into rabbits was excreted in the urine within 24 hours. However, without additional data (content of triclopyr in tissues, excretion in feces, quantities remaining on the skin-site of applications, etc.) the conclusions drawn by the authors are not totally acceptable.

Conclusions:

Under the conditions of this study the dermal absorption of triclopyr in female rabbits through the abraded skin appears to be very low accouting only for less than 1.5 percent of the total dose applied based on triclopyr excretion in the urine within 24 hours postapplication.

Classification:

The present study is classified as Core-Supplementary for the following deficiencies:

1. Only two female and no male animals were used.

2. The authors did not explain how they were able to apply 28 to 30 mL of triclopyr suspension onto the shaved skin without losing most of it as runoff.

3. The authors did not estimate the total amount of triclopyr absorbed (as percent of the dose) since they did not assay for triclopyr excreted in feces or retained in tissues and they did not account for triclopyr remaining on the application site.

4. The stability of triclopyr in demineralized water at room temperature for up to 24 hours was not reported.

TABLE 1 - URINARY EXCRETION OF TRICLOPYR BY TWO FEMALE RABBITS AFTER ONE SINGLE DERMAL APPLICATION ON THE ABRADED SKIN.

period	volume of sterilized urine	amount of triclopyr excreted per time interval	amount in per cent of administered dose (2 g/kg)	rate of urinary excretion of unchanged triclopyr
	rabbit no. 200 (body weight: 2.42 kg)			
0- 4 h	27 ml	0.8 mg	0.01 %	0.2 mg/h
4- 8 h[1]	0 ml	0 mg	0 %	0 mg/h
8-13 h	34 ml	19.4 mg	0.34 %	3.9 mg/h
13-24 h	73 ml	45.9 mg	0.81 %	4.2 mg/h
24-27 h	3 ml	0.3 mg	0.01 %	0.2 mg/h
total				
0-24 h	134 ml	66.2 mg	1.17 %	
	rabbit no. 203 (body weight: 2.91 kg)			
0- 4 h	81 ml	8.3 mg	0.11 %	1.4 mg/h
4- 8 h	22 ml	14.1 mg	0.24 %	3.3 mg/h
8-13 h	27 ml	17.2 mg	0.30 %	3.3 mg/h
13-24 h	97 ml	24.3 mg	0.42 %	2.2 mg/h
24-27 h	14 ml	4.3 mg	0.08 %	1.3 mg/h
total				
0-24 h	227 ml	63.1 mg	1.07 %	

1) after 4 hours no urine could be obtained from rabbit no. 200, ~~was 0-8~~

Subject: Study on the Percutaneous Absorption of Triclopyr by the Rabbit. Part II: Single Treatment of Intact Skin. (Note: Study probably conducted under OECD Guidelines.)

Test Material: Triclopyr, 99% ai (3,5,6-trichloro-2-pyridyloxyacetic acid).

Accession Number: 259680

Sponsor: Dow Chemical Company Ltd., Norfolk, U.K.

Testing Facility: Netherlands Organization for Applied Scientific Research, TNO.

Report Number: V81.422/212062 (Project No. 881/2062)

Testing Period: Not specified (possibly April 1981)

Report Submitted to Sponsor: November 1981

Methods and Materials:

Two female New Zealand White albino rabbits, weighing 2.6 and 3.2 kg were used in this study (age and source of animals were not specified). Before the initiation of the study the animals had free access to standard laboratory diet (name of diet not specified) and tap water. The day before treatment approximately 20 percent of the body surface was shaved with electric clippers (trunk area) and (on day of treatment) the shaved area was wrapped in polyethylene foil. The animals were then held in restraining boxes and their bladders were emptied using a catheter. Triclopyr, 99% pure (AGR 134832), was dissolved in demineralized water to give a 20 percent (w/v) suspension. Then 26 or 32 mL of the 20 percent suspension (corresponding to 2 g triclopyr/kg body weight and a volume of 10 mL/kg body weight) were injected through the polyethylene foil onto the shaved skin and the animals were kept in the restraining boxes for 24 hours (after application) without food or water. At various intervals (during the 24-hour observation period) the bladders were emptied using a catheter and the urine collected was later analysed for triclopyr content using the EC-GLC procedure. Triclopyr recovery (from spiked samples) was reported to be greater than 91 percent.

Results:

The present study has investigated the dermal absorption of triclopyr in rabbits when applied to the intact skin at a level of 2 g/kg body weight. Data shown on Table 1 (abstracted

from the original report) indicate that approximately 1.2 percent of the total dose applied to the skin is excreted in the urine within 24 hours after application. The rate of excretion is slow throughout the 24-hour period and is obviously limited by the amount of triclopyr absorbed through the intact skin. As was the case with a previous study on the percutaneous absorption of triclopyr in rabbits by the abraded skin (Report Vol.322), the authors concluded that absorption of triclopyr through the intact skin is very low. However, in both studies the authors based their conclusion on the assumption that almost all triclopyr absorbed through the skin is excreted in the urine as parent compound within 24 hours (see also DER on "urinary excretion of intravenously injected triclopyr by the rabbit," Report No. Vol.3.2). This reviewer believes that the authors have not shown conclusively that (1) over 95 percent of the triclopyr dose injected intravenously in rabbits is excreted in urine (see comments in corresponding DER; Report No. Vol.312), and (2) only less than 1.5 percent of the applied dose is absorbed through the abraded (Report No. Vol.322) or intact (present study) skin in rabbits (since the amounts excreted in feces, retained in tissues, or remaining on the application site were not measured).

Conclusions:

Under the conditions employed in conducting this study the absorption of triclopyr through the intact skin of female rabbits, based on triclopyr excretion in the urine, is very low and represents less than 1.5 percent of the total applied dose.

Classification:

The present study is classified as Core-Supplementary due mainly to the following deficiencies:

1. Only two female animals and no male animals were used.

2. The authors did not explain how they were able (and how long it took) to apply up to 12 mL (10 mL/kg body weight) of triclopyr suspension on the shaved skin without losing most of it in the process (as runoff).

3. The authors did not estimate the total amount of triclopyr absorbed (as percent of the total dose) since they did not assay for quantities of triclopyr excreted in feces, retained in tissues, or remaining on the application site.

4. The stability of triclopyr in demineralized water at room temperature for up to 24 hours, was not reported.

5. No mention was made by the authors in any of the three aforementioned studies (Report No. Vol.III; Report No. Vol.IIA; present study, Report No. Vol.4A2) as to the metabolism of triclopyr by female rabbits.

TABLE 1 - URINARY EXCRETION OF TRICLOPYR BY TWO FEMALE RABBITS AFTER ONE SINGLE DERMAL APPLICATION ON THE INTACT SKIN.

period	volume of catheterized urine	amount of triclopyr excreted per time interval	amount in per cent of administered dose (2 g/kg)	rate of urinary excretion of unchanged triclopyr
	rabbit no. 304 (body weight: 2.6 kg)			
0- 4 h	12 ml	8.4 mg	0.32 %	1.6 mg/h
4- 8 h	20 ml	16.2 mg	0.31 %	4.0 mg/h
8-13 h	30 ml	19.0 mg	0.3 %	3.8 mg/h
13-24 h	56 ml	40.3 mg	0.78 %	3.7 mg/h
24-27 h	5 ml	2.1 mg	0.04 %	0.7 mg/h
total				
0-24 h	118 ml	82.1 mg	1.58 %	
	rabbit no. 343 (body weight: 3.3 kg)			
0- 4 h	9 ml	3.2 mg	0.05 %	0.8 mg/h
4- 8 h	28 ml	13.7 mg	0.21 %	3.4 mg/h
8-13 h	31 ml	13.8 mg	0.21 %	3.3 mg/h
13-24 h	48 ml	22.8 mg	0.34 %	2.1 mg/h
24-27 h	8 ml	2.4 mg	0.04 %	0.8 mg/h
total				
0-24 h	112 ml	53.3 mg	0.87 %	

Subject: Evaluation of Triclopyr in the Mouse Bone Marrow Micronucleus Test

Test Material: Triclopyr (3,5,6-trichloro-2-pyridyloxyacetic acid) 96.1% ai (Lot # AGA-204229)

Accession Number: 073873

Sponsor: Dow Chemical, U.S.A.; Midland, Michigan

Testing Facility: Dow Chemical U.S.A., Lake Jackson Research Center, Freeport, Texas

Study Number: TXT:K-042005-(28)

Testing Period: December 1984

Report Submitted to Sponsor: May 1985

Materials and Methods:

Male and female CD-1 (ICR) BR mice (purchased from Charles River, Wilmington, MA) approximately 8 weeks old, were used in this study. Upon arrival all animals were examined for their health status and kept individually in wire-bottom cages with unique identification numbers. The mice were acclimated for 2 weeks in a room where temperature was maintained at 72 ± 5 °F, relative humidity between 40 to 60 percent, 12 air changes per hour, and a 12-hour light cycle. All animals were offered Purina Certified Rodent Chow #5002 and water ad libitum.

The mice were weighed and randomly divided into 4 groups of 10 animals/group/sex and administered orally (by gavage) a single dose of either corn oil (negative control) at 0.2 mL/10 g body weight or triclopyr (dissolved in corn oil) at 30, 90, or 280 mg/kg body weight in a total volume of 0.2 mL aliquot/10 g mouse. A fifth group (five males and five females) of mice was administered orally cyclophosphamide at 120 mg/kg in a volume of 0.5 mL/10 g body weight and served as positive control. The high-dose level of triclopyr used in the study (280 mg/kg) represented 60 percent of the LD_{50} (LD_{50} = 471 mg/kg for mice) of triclopyr in mice. The selected dose level of cyclophosphamide, 120 mg/kg, was based according to the sponsor, on earlier studies (unpublished data) carried out in the same laboratory which showed that this dose induced the formation of micronuclei. Triclopyr solutions (in corn oil) and cyclophosphamide solutions (in distilled water) were used within 2 hours after preparation. Triclopyr and cyclophosphamide concentrations were verified by high-pressure liquid chromatography (HPLC) at the sponsor's laboratory.

Animals were sacrificed (by cervical dislocation) at two time intervals as follows:

- At 24 hours postadministration: five male and five female animals from the negative control group (vehicle control), triclopyr-treated groups and positive control group.

- At 48 hours postadministration: five male and five female animals from the negative control group and triclopyr-treated groups.

Bone marrow samples were obtained from both femurs of each mouse using a syringe containing 0.3 mL of fetal calf serum. The syringe contents were transferred to a centrifuge tube (containing approximately 0.3 mL of calf serum), resuspended, and centrifuged at 1000 rpm for 5 minutes. The supernatant fraction was discarded and the pellet was resuspended and cell smears were prepared on microscope slides. The slides were air-dried and stained using a modified Giemsa method of Gollapudi and Kamra (1979). Slides were coded and scored blindly. From each animal, 1000 polychromatic erythrocytes (PCE) were examined and the number of micronucleated polychromatic erythrocytes (MN-PCE) was recorded. Micronuclei were identified as darkly stained bodies with sharp contours and varying shapes (Schmid 1976). The ratio of polychromatic erythrocytes (PCE): normochromatic erythrocytes (NCE) was determined by examining approximately 200 erythrocytes. The ratio was expressed as $\frac{PCE}{PCE + NCE} \times 100$.

Statistical Analysis:

The frequencies of micronucleated polychromatic erythrocytes (MN-PCE) were analysed by constructing two-dimensional contingency tables of dose x frequency class (number of animals with 0, 1, 2, etc., MN-PCE). The total Chi-square was partitioned into components of interest. Specifically, statistics were generated to test the two global hypotheses of (1) no differences in average scores among the doses (difference test), and (2) no linear trend of increasing scores with increasing dose (order test) (Bhaghkar 1968). If either statistic was found to be significant at alpha=0.01, then pairwise (control vs. treatment) tests were also performed for each dose level and evaluated at alpha=0.01.

Preliminary examination of this type of data has shown that MN-PCE frequencies can be described very well by a Poisson distribution. Under this interpretation, the above tests can be viewed as tests for dose-related changes in the Poisson parameter, i.e., in the expected number of MN-PCE per observational unit (animal).

A quality assurance statement and a protocol were signed and presented.

Results:

Triclopyr and cyclophosphamide concentrations in the dosing solutions as determined by HPLC were found by the sponsor to be closely comparable to the target concentrations (ranging from 96 to 104 percent of the target concentration).

The results of the present study indicate that the frequency of MN-PCE in the triclopyr-treated groups (low, medium, and high-dose groups) was not significantly different than the frequency of MN-PCE in the negative control group in both sexes of mice (Tables 1 and 2, abstracted from the original report). The results were similar between the 24-hour and the 48-hour time points.

Other parameters measured such as ratio of PCE to NCE (expressed as % PCE in Tables 1 and 2) did not indicate changes attributable to triclopyr toxicity or triclopyr interference with cellular proliferation. Again these parameters were similar in male and female mice at both time points examined. On the other hand, animals treated with cyclophosphamide (the positive control group), showed a statistically significant increase in the frequencies of MN-PCE in the bone marrow of both sexes (Tables 1 and 2). These results also show that the ratio of PCE to NCE (% PCE) was significantly lower in the males and females of the positive control group as compared to the corresponding negative control group (Tables 1 and 2).

Discussion:

Data obtained from the present study indicate that triclopyr does not significantly increase the frequency of micronuclei in PCE in the bone marrow of male or female mice at either the 24-hour or the 48-hour posttreatment sampling intervals. It is not clear from the available data, however, whether the high-dose level selected (280 mg/kg) was adequately high to be considered as the MTD. Although the authors reported that they based the dose selection on a published LD_{50} value of triclopyr in mice of 471 mg/kg (Hanck et al. 1979), 60 percent of the LD_{50} value (280 mg/kg, the high dose tested) did not appear to cause any toxic effects in the treated mice. Thus, body weights changes were not significantly different between the triclopyr-treated and the negative control (vehicle-treated) groups. As no other data were reported pertaining to triclopyr toxicity, this reviewer believes that the high dose tested was not adequately high.

With the exception of the dose selection, all other aspects of the protocol employed were acceptable and all parameters measured were adequate in demonstrating an effect of the test chemical if such an effect were present. The sensitivity of the assay used to detect genotoxicity was sufficiently demonstrated by the statistically significant increase (over eightyfold in males and over thirtyfold in females) in cells with micronuclei in the positive control group (cyclophosphamide) compared to the vehicle control group (corn oil).

Conclusions:

Under the conditions of the mouse bone marrow micronucleus assay, bromopyr did not induce a significant increase in the frequencies of micronucleated bone marrow polychromatic erythrocytes when administered as a single dose to male and female mice at dose levels of 45, 90, or 180 mg/kg body weight.

Classification:

Unacceptable (due to the selection of insufficient doses to induce either clinical toxicity or cytotoxicity as the target).

TABLE 2

SUMMARY OF THE DATA ON THE FREQUENCIES OF MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES (MN-PCE) IN THE BONE MARROW OF MALE MICE TREATED WITH TRICLOPYR OR CYCLOPHOSPHAMIDE (CP)

	24 h Sacrifice				48 h Sacrifice			
Dose	NA	PCE Examined	MN-PCE[b]	% PCE	NA	PCE Examined	MN-PCE[b]	% PCE
0 mg/kg Negative Control	5	5000	0.2 ± 0.4	66.9 ± 7.8	5	5000	0.2 ± 0.4	70.1 ± 4.1
20 mg/kg Triclopyr	5	5000	0.4 ± 0.9	70.8 ± 3.9	5	5000	1.0 ± 1.0	72.2 ± 7.7
80 mg/kg Triclopyr	5	5000	0.4 ± 0.9	74.9 ± 7.8	5	5000	0.2 ± 0.4	67.9 ± 9.0
280 mg/kg Triclopyr	5	5000	0.8 ± 0.8	69.9 ± 9.4	5	5000	0.4 ± 0.8	68.4 ± 8.9
120 mg/kg CP Positive Control	5	5000	51.0[c] ± 13.6	44.8 ± 10.0	ND[d]			

[a]N = number of mice.

[b]Data are means and standard deviations.

[c]Significantly different from negative control.

[d]ND = not done.

TABLE 8.

SUMMARY OF THE DATA ON THE FREQUENCIES OF MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES (MN-PCE) IN THE BONE MARROW OF FEMALE MICE TREATED WITH TRICLOPYR OR CYCLOPHOSPHAMIDE (CP)

	24 h Sacrifice				48 h Sacrifice			
Dose	No	PCE Examined	MN-PCE[a]	% PCE	No	PCE Examined	MN-PCE[a]	% PCE
0 mg/kg Negative Control	5	5000	0.4 ± 0.9	69.7 ± 4.8	5	5000	0.8 ± 1.3	72.8 ± 4.9
25 mg/kg triclopyr	5	5000	0.2 ± 0.4	71.4 ± 9.8	5	5000	0.6 ± 0.9	64.6 ± 9.3
90 mg/kg triclopyr	5	5000	1.4 ± 1.7	78.9 ± 3.0	5	5000	0.2 ± 0.4	71.1 ± 7.6
200 mg/kg triclopyr	5	5000	0.8 ± 1.3	72.9 ± 8.1	5	5000	1.2 ± 0.8	74.2 ± 3.8
120 mg/kg CP Positive Control	5	5000	47.0[c] ± 8.2	49.3 ± 6.5	ND[d]			

[a]N = number of mice.

[b]Data are means and standard deviations.

[c]Significantly different from negative control.

[d]ND = not done.

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